Dissociation of nucleoprotein complexes by chaotropic salts

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The effect of various anions in destabilizing yeast nucleoprotein complexes followed the order $F^- < Cl^- < Br^- < ClO_4^- \simeq Cl_3CCOO^-$. Treatment of yeast nucleoproteins with 0.5 M $NaClO_4$ resulted in removal of 80% of RNA. Based on the results, a simple method for effective separation of RNA from ribosomal particles is proposed and the mechanism of RNA dissociation by anions is also discussed.

Yeast *Ribosome* *Nucleoprotein* *Chaotropic salt* *Dissociation*

1. INTRODUCTION

The isolation, purification and characterization of ribosomal proteins and enzymes has been difficult because of interference from contaminant nucleic acids in these preparations. Conventionally, the proteins are detached from ribosomes by treatment with urea, detergents [1], phenol [2], or concentrated acetic acid [3]. While these methods are effective in isolating the proteins, they cause irreversible denaturation of the proteins and thus impair subsequent biochemical characterization. LiCl extraction of ribosomal proteins has been shown to yield proteins which still retained their 'native' structure [4–7]. These procedures employ high [LiCl] alone or in combination with other denaturing agents such as urea. Although the exact mechanism by which LiCl induces dissociation of proteins from ribosomes is not well established, it is generally believed that it is mainly via neutralization of the electrostatic interactions between proteins and RNA. Such an explanation fails to answer the question as to why 2–4 M LiCl is needed to neutralize the non-specific electrostatic interactions. Furthermore, it does not adequately explain why different protein fractions are preferentially extracted at different [LiCl] [7]. This suggests that although electrostatic interactions may play a major role in protein–RNA interactions in ribosomes, there may be some other types of interactions which may play an equally important role in the stability of nucleoprotein complexes in ribosomes. It is known that apart from affecting electrostatic interactions at high concentrations, LiCl may also act as a chaotropic agent [8]. At high concentrations, LiCl affects hydrophobic interactions and induces destabilization of oligomeric structures by changing the solvent structure [8]. It may be possible that the LiCl-induced dissociation of proteins from ribosomes may be both due to a charge neutralization mechanism and destabilization of hydrophobic interactions between protein subunits which may be an indirect result of solvent destructuring.

To understand the mechanism by which salts dissociate proteins from ribosomes, we studied the effects of other chaotropic anions on the dissociation of RNA from yeast nucleoprotein complexes. We observed that the effectiveness of various anions on the dissociation of RNA follow the classical Hofmeister series [9] which suggest that apart from electrostatic effects, the chaotropic anions may also dissociate nucleoprotein complexes via destabilization of hydrophobic interactions by altering the structure of solvent water.

2. MATERIALS AND METHODS

Brewer's yeast (*Saccharomyces carlbergensis*) was obtained from Genesee Brewing Co. (New

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York). The yeast samples were washed 3 times with cold distilled water before use. All chemicals used in this study were of analytical grade.

2.1. *Extraction of yeast nucleoproteins*

Yeast cells were disrupted using a Dyno-Mill (Type KDL, Willy A. Bachofen, Manufacturing Engineers, Basel) at 5°C. Under the conditions employed >95% of the yeast cells were distrupted as observed under a phase contrast microscope.

The pH of the homogenized yeast cell suspension was adjusted to 9.0 and stirred for 30 min at 5°C to solubilize the nucleoprotein. The solution was then centrifuged at 15000 × *g* for 30 min at 2°C to remove the cell wall, undisrupted cells and other insoluble materials.

The nucleoproteins from the supernatant were isolated by isoelectric precipitation at pH 4.2 followed by centrifugation. The precipitate was dissolved in distilled water at pH 9.0 and freeze dried. The RNA dissolved in distilled water at pH 9.0 and freeze dried. The RNA content of the nucleoprotein was about 12.5–14.5% on dry weight basis.

2.2. *Protein and RNA estimations*

The protein content was determined by Biuret method. The RNA content of the nucleoproteins was determined by the Orcinol method [10]. According to this method, a weighted amount of the nucleoprotein was incubated in 0.5 M phosphoric acid at 37°C for 2 h with shaking. The solution was centrifuged and the RNA content of the supernatant was estimated spectrophotometrically with orcinol reagent. Purified yeast RNA (Sigma, St Louis MO) was used as standard.

2.3. *Dissociation of RNA*

The effect of chaotropic salts on the dissociation of RNA from the yeast nucleoprotein complex was studied as follows: To aliquots of nucleoprotein solutions (30 mg/ml, pH 9.0), the salts were added at various concentrations. After dissolving the salts, the pH of the protein solutions was decreased to 4.2 with 2 N HCl to precipitate the protein. The residual salts in the precipitates were removed by washing the precipitates twice with 10 vol. distilled water at pH 4.2. The precipitates were then dissolved in distilled water at pH 9.0 and lyophilized. The RNA content of these treated samples was determined.

To determine the effects of temperature on $NaClO_4$-induced dissociation of RNA from the nucleoprotein complex, aliquots of yeast nucleoprotein solutions (30 mg/ml) at pH 9.0 were immersed in water baths at various temperatures. After the protein solutions attained the specific temperature (1.5 min), known quantities of $NaClO_4$ were added (0.5 M). The pH of the solution was immediately adjusted to 4.2 to precipitate the proteins. The samples were centrifuged at the specified temperatures and supernatants were recovered. The precipitates were then washed twice with distilled water (pH 4.2) at the respective temperatures to remove the residual $NaClO_4$. The precipitates were dissolved in water at pH 9.0, lyophilized and the RNA content of the lyophilized samples determined.

3. RESULTS

The effects of various salts on the removal of RNA from yeast nucleoprotein complex is shown in fig.1. The RNA content of the isolated protein decreased with increasing salt concentration. At lower salt concentrations, the effectiveness of the salts in decreasing the RNA content followed the Hofmeister series [11]; i.e., $F^- < Cl^- < Br^- < ClO_4^- \simeq Cl_3CCOO^-$. For example, at 0.5 M,

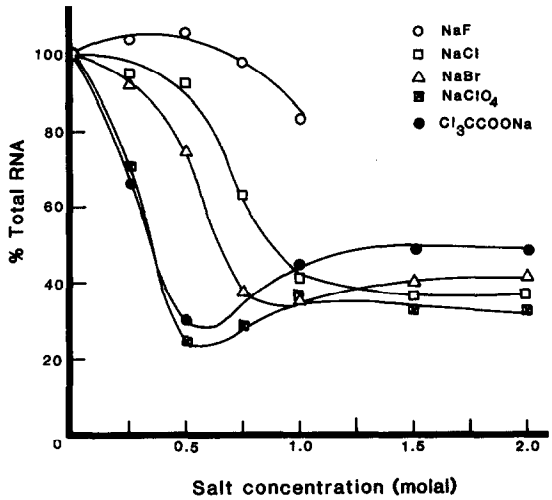

Fig.1. Effects of chaotropic salts on the separation of RNA from yeast nucleoprotein complex at 25°C; nucleoprotein was 30 mg/ml; initial RNA content of the nucleoprotein was ~12.5%.

$NaClO_4$ removed about 80% of RNA initially present in the nucleoprotein complex whereas NaCl and NaBr removed only about 10% and 25%, respectively. In the case of NaF, at 0.5 M, the RNA content was higher than the control. Treatment with $NaClO_4$ and Cl_3CCOON_a above 0.5 M increased the RNA content whereas NaCl and NaBr decreased the RNA content up to 1 M (fig.1).

The magnitude of the effect of anions on the dissociation of RNA was found to be independent of the initial RNA content of the nucleoprotein complex. For example, irrespective of the initial RNA content, treatment of the nucleoprotein with 0.5 M $NaClO_4$ resulted in extraction of ~80% of the total RNA. Continuous treatment of the nucleoprotein with 0.5 M $NaClO_4$ resulted in further reduction in the RNA content (fig.2). After four treatments with 0.5 M $NaClO_4$, about 98–99% of the RNA originally present in the nucleoprotein was removed.

On treatment with $NaClO_4$ and Cl_3CCOON_a, the RNA content of the pretreated protein decreased up to 0.5 M and then increased above this salt concentration (fig.1). One reason may be the salting-out of the dissociated RNA above 0.5 M $NaClO_4$ at pH 4.2. However, experiments with purified yeast RNA (obtained from Sigma Co.) did not reveal any salting-out effect by $NaClO_4$ at the concentration of RNA found in yeast protein. After treatment with $NaClO_4$ with decreasing RNA content, the protein increasingly precipitated at pH 8.0 (fig.2). Similar observations were also made during hydrolysis of RNA by endogenous ribonuclease under conditions which inhibited the endogenous protease activity [12]. Insolubilization and aggregation of the protein may reflect exposure of hydrophobic regions which were originally buried inside the ribosomal structure and subsequent protein–protein interaction.

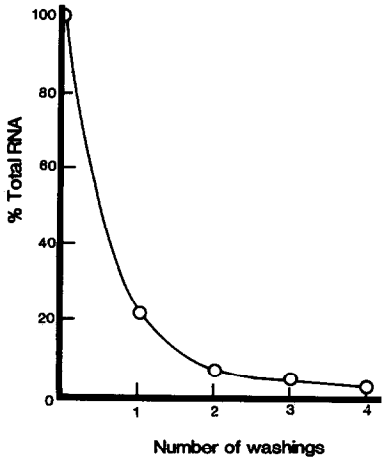

Fig.2. Effect of multiple washing with 0.5 M $NaClO_4$ on the RNA content of the yeast nucleoprotein; initial RNA content of the nucleoprotein was ~12.5%.

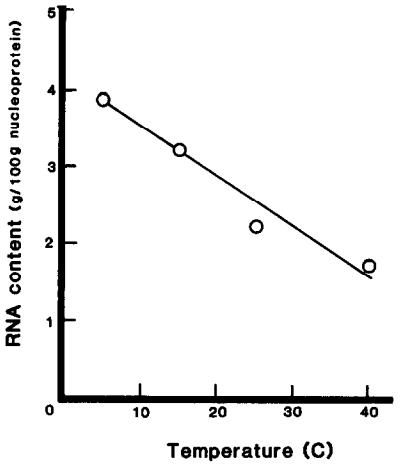

Fig.3. Effect of temperature on the removal of RNA from yeast nucleoprotein complex by $NaClO_4$ at 0.5 M; initial RNA content of the nucleoprotein was ~12.5%.

The effect of temperature on the RNA content of the nucleoprotein treated with 0.5 M $NaClO_4$ is shown in fig.3. The RNA content decreased at high temperatures. Since the optimum pH of endogenous ribonuclease is 5.8 and has negligible activity at pH 9.0, the observed decrease in the RNA content with temperature cannot be attributed to increased endogenous ribonuclease activity. The decrease in RNA content with increasing temperature indicates that the nucleoprotein complex was more destabilized at higher temperatures in the presence of 0.5 M $NaClO_4$.

4. DISCUSSION

The interaction between protein and nucleic acids in nucleoprotein complexes is complex and may involve hydrogen bonding, electrostatic and hydrophobic interactions [14]. Since about 80% of total RNA in microbial cells are in the form of ribosomal RNA, it may be pertinent to consider the salt-

induced dissociation of RNA from the point of their effect on ribosome structure. The results presented here show that the dissociation of proteins from nucleoprotein complexes is influenced by the salt concentration as well as the type of anion. The effectiveness of various anions in inducing dissociation of RNA followed the classical Hofmeister series [11]; i.e., $F^- < Cl^- < Br^- < ClO_4^- \equiv Cl_3CCOO^-$. This series basically reflects the relative effectiveness of anions in causing destabilization of proteins [11]. The destabilizing effects of anions on proteins has been attributed to their effect on water structure which in turn affect hydrophobic interactions [8].

Although electrostatic interactions may play a major role in stabilizing the nucleoprotein complexes in ribosomes, the role of hydrophobic interactions in stabilizing the electrostatic interactions cannot be neglected. It has been shown that most of the ribosomal proteins contain ~42–52% apolar amino acid residues indicating that they are highly hydrophobic [3]. The arrangement of these proteins in the ribosomal structure conceivably creates non-polar regions. The subsequent formation of electrostatic bonds between lysyl and phosphate groups at this local low dielectric region should exhibit higher energy than the electrostatic bonds formed in the relatively more polar regions [14]. In other words, within the nucleoprotein complex there may be several classes of electrostatic bonds having different bond energies depending on the local dielectric conditions. Such an argument, in principle, is consistent with the observation that different ribosomal proteins are preferentially dissociated at different [LiCl] [7]. Thus the proteins that are detached from ribosomes at 1 M LiCl may be located in a relatively more polar environment than the proteins which are detached from ribosomes at 2 M or 4 M LiCl. This may indicate that to destabilize the electrostatic bonds located in relatively low dielectric environment created by the arrangement of the proteins in ribosomes, it may be necessary first to destabilize the ribosome structure. Destabilization of the ribosomal protein–protein interactions is conceivably mediated via changes in the solvent structure at high LiCl concentrations. Under such conditions, the electrostatic bonds which were previously in the low dielectric field may become more susceptible to charge neutralization by the salts.

In [14], it was pointed out that the conformation of the ribosome is an important factor in the detachment of ribosomal proteins by NaCl. Thus, slight destabilization or changes in the conformation of ribosome influences the effectiveness of a particular salt in dissociating the ribosomal proteins. Furthermore, the secondary structure of ribosomal RNA has been shown to play an important role in its interaction with ribosomal proteins [16]. Changes in the secondary structure of the 16 S RNA resulted in the loss of its capacity to interact with several ribosomal proteins [16]. In [17], several chaotropic salts affected the melting temperature of DNA; the effectiveness of the salts in destabilizing the helical structure of DNA followed the Hofmeister series. In [18], neutral salts affected the solubility of nucleotides and their effectiveness followed the Hofmeister series. The observed effects of chaotropic salts on the dissociation of RNA reported here may also be partly related to the destabilization of the secondary structure of ribosomal RNA.

These results suggest that the proteins from nucleoprotein complexes may be separated by relatively mild treatment with the chaotropic salts such as $NaClO_4$ or NaSCN. Although the electrostatic interactions may play a major role in the stabilization of nucleoprotein complexes, other types of interactions may also contribute significantly to the stability of such complexes. Similar studies with ribosomes may reveal some insight into the organization of protein and RNA in ribosomal structure.

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